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Pax-2, Kidney Development, and Oncogenesis

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The development of a complex tissue from a few simple precursor cells requires the precise activation and repression of tissue-specific genes that determine cell lineages, tissue patterning, and cellular proliferation. In the kidney, a number of recently identified genes are critical for normal development. Among these, the *Pax-2* gene encodes a transcription factor that is expressed in the ureter bud, in the induced kidney mesenchyme, and in the progenitor cells of the glomerular and tubular epithelium. Although the differentiation of the renal epithelium requires *Pax-2* function, failure to suppress the gene in mature epithelium is detrimental to normal renal function. Recent, data suggest that the Wilms' tumor-suppressor gene *WT1* can down-regulate *Pax-2* expression, consistent with high levels of *Pax-2* in Wilms' tumors. Additional studies suggest that reactivation of this developmental regulator can contribute to a variety of other renal diseases.

Key words: kidney, *Pax-2*, development, transcription, Wilms' tumor

INTRODUCTION

The developing kidney is an excellent model for basic developmental studies on inductive interactions, epithelial cell polarization, and tissue remodeling. Since the introduction of kidney organ culture and the trans-filter induction method [1], the conversion of the metanephric mesenchyme in response to induction by the ureteric bud has been described to detail [2,3]. However, less is known about the underlying genetic mechanisms that specify epithelial cell differentiation and pattern formation in the kidney. Through the convergence of *Drosophila*, mouse, and human developmental genetics, a number of critical genes have been identified recently that function at key stages of early kidney development. These would include the Wilms' tumor-suppressor gene, *WT1* [4], the receptor-type tyrosine kinase and proto-oncogene, *c-ret* [5], the secreted signaling protein, *wnt-4* [6], and the transcription factor *Pax-2* [7]. Strikingly, these important developmentally expressed genes also play critical roles in disease processes of the kidney and other tissues.

PAX-2 IS REQUIRED FOR EARLY EPITHELIAL DIFFERENTIATION

Development of the adult kidney begins when the ureteric bud, an outgrowth of the wolffian duct, contacts a group of cells called the metanephric mesenchyme in the posterior intermediate mesoderm. In the mouse, this occurs at 11 days gestation and results in a reciprocal inductive event [2]. The ureteric bud induces the mesenchyme to proliferate and convert to an epithelium and generate most of the tubular epithelium of the nephron, whereas the mesenchyme induces the ureteric bud to proliferate and branch, thus forming the collecting ducts. One of the earliest known markers activated in the induced mesenchyme is the transcription factor *Pax-2* [8,9]. The *Pax-2* gene is a member of a multigene family that includes the genes responsible for such developmental abnormalities as human aniridia [10] and Waardenburg syndrome [11,12] and the mouse mutation undulated [13]. The salient features of the *Pax-2* gene are summarized in Table I.

In the developing mammalian kidney, the expression pattern of *Pax-2* suggests an early function in the differentiation of the renal epithelium (Fig. 1). During a well-defined sequence of morphogenic events, *Pax-2* expression is observed in the condensing mesenchyme and renal vesicle [8,9], a spherical epithelial cyst of poorly differentiated and proliferating cells. In mutant mice that fail to induce the mesenchyme because of ureter bud defects, mesenchymal *Pax-2* expression is not detected [23]. Thus, *Pax-2* activation requires induction and is not an inherent property of the metanephric mesenchyme. Expression persists in the comma-shaped body and in the distal two thirds of the s-shaped body. However, *Pax-2* repression then becomes evident in the proximal part of the s-shaped body from where the podocyte cells originate. Finally, *Pax-2* expression is down-regulated in all of the tubular epithelium of the nephron, although expression is still detected in the collecting ducts. In transgenic mice that continue to express *Pax-2* in the kidney, using a heterologous promoter, severe kidney abnormalities are observed,

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TABLE I. Features of the *Pax-2* Gene

Map position	Mouse chromosome 19 [14]
	Human chromosome 10q24-25 [15]
Protein	415 and 392 amino acids, generated by alternate splicing [8, 9]
DNA binding	Divergent consensus, T-GTCAYGCRTGA [15, 16]
Neural expression	Neural folds, midbrain/hindbrain junction
	Neural tube, differentiating cells of the intermediate zone
	Ventral half of the optic cup and stalk
	Medial two thirds of the otocyst [17, 18]
Kidney expression	Pro- and mesonephric ducts, ureter bud
	Mesonephric tubules
	Induced metanephric mesenchyme, comma-shaped body
	Distal two thirds of s-shaped body
	Collecting ducts [8, 9]
Disease expression	Wilms' tumor [9, 15], RCC. [19]
	Polycystic kidney [20]
Mutations	Human, partial deletion of one allele [21]
	Hypoplastic kidneys, viscouretal reflux, ocular colombomas
	Mouse, 7 cM deletion spanning entire locus [22]
	Homozygotes—early embryonic lethal
	Heterozygotes—small kidneys, attenuated nephrogenic zone reduced numbers of retinal ganglion cells

including the formation of microcysts, proteinurea, and loss of podocyte foot processes [24]. These data suggest that *Pax-2* repression is necessary for terminal differentiation of at least some renal epithelial cells.

Mutations at the *Pax-2* locus have now been described in both mouse and humans. The *Krd* mouse has a large 7 cM deletion spanning the entire *Pax-2* locus [22]. Although this deletion could encompass hundreds of genes, the phenotype of the heterozygous *Krd* mice is consistent with the *Pax-2* expression pattern in the eye and kidney and the semidominant nature of other *Pax* gene mutations. The affected mice have aplastic and hypoplastic kidneys with a reduced number of nephrons, the nephrogenic zone appears attenuated, and cyst formation is frequent. In the eye, the retinal ganglion layer has reduced cell numbers and disrupted laminar architecture. In the human mutation, a single nucleotide deletion results in a frame shift of the coding region just downstream of the DNA-binding domain [21]. The phenotypes of individuals with only one functional copy of the *Pax-2* gene include renal hypoplasia, vesicoureteral reflux, and optic nerve colombomas. The mutations clearly implicate the *Pax-2* gene in the regulation of renal epithelial cell proliferation and differentiation. Previous in vitro experiments using antisense methods to inhibit *Pax-2* function are consistent with the mutant studies and demonstrate the effect of *Pax-2* is at the earliest phase of epithelial differentiation, the condensation of the mesenchyme [7].

PAX-2 AND ONCOGENESIS

How developmental control genes that regulate cell proliferation and differentiation can be reactivated in adult cells and contribute to the initiation and progression of tumors is central to understanding molecular mechanisms of transformation. Because of the embryonic origins of Wilms' tumor, it was not surprising that the *Pax-2* gene is expressed in the epithelial component of the tumor [9,15]. Although this persistent *Pax-2* expression may be more a reflection of the developmental origins of the tumor, failure to suppress *Pax-2* may be a direct consequence of genetic lesions. In fact, recent evidence suggests that the Wilms' tumor-suppressor gene *WT1* can repress *Pax-2* expression at least in part of the developing nephron [25]. The proximal portion of the s-shaped body, the progenitor cells of the podocytes, is the first region to suppress *Pax-2* expression during nephrogenesis. Strikingly, these podocyte progenitors express high levels of the *WT1* protein that is capable of binding *Pax-2* regulatory elements and that can suppress transcription [25]. The zinc-finger domain of *WT1* can bind to at least three elements located in the 5′ untranslated leader sequence of the *Pax-2* gene that are in close agreement with known *WT1* consensus-binding sites [26]. These *WT1*-binding elements can repress transcription in tissue culture cells when cotransfected with increasing amounts of a *WT1*-expressing vector. Using sequences upstream of the *Pax-2* transcription start site from either −7000 or −4300 to position +675, expression of a chloramphenicol acetyltransferase reporter gene is reduced more than fivefold with increasing amounts of *WT1*. If the *WT1*-binding sites from the 5′ leader sequences are inserted between a heterologous promoter and the reporter gene, expression is reduced more than 10-fold. These data suggest that *WT1* can repress *Pax-2* transcription during the normal course of kidney development.

One of the most common adult malignancies is renal cell carcinoma (RCC), which occurs both sporadically and with a genetic predisposition. Based on ultrastructural and immunocytochemical analyses, RCC is generally thought to arise from the epithelium of the proximal kidney tubules [27–29]. Recently, the gene for von Hippel-Lindau (VHL) disease, a hereditary cancer syndrome predisposing affected patients to RCC as well as other tumors, was cloned [30]. *VHL* mutations were identified in nearly 60% of the sporadic, nonpapillary RCC tumors and derived cell lines examined [31,32]. In addition, the normally constitutive transcription of the *VHL* gene was silenced by hypermethylation in another 20% of RCC tumors [33]. Thus, *VHL* plays an important role in the origins of RCC, although the nature of the VHL protein and its mechanism of action remain unclear.

Given the presumed origin of RCC, the proximal tubule epithelium, the expression of *Pax-2* was examined

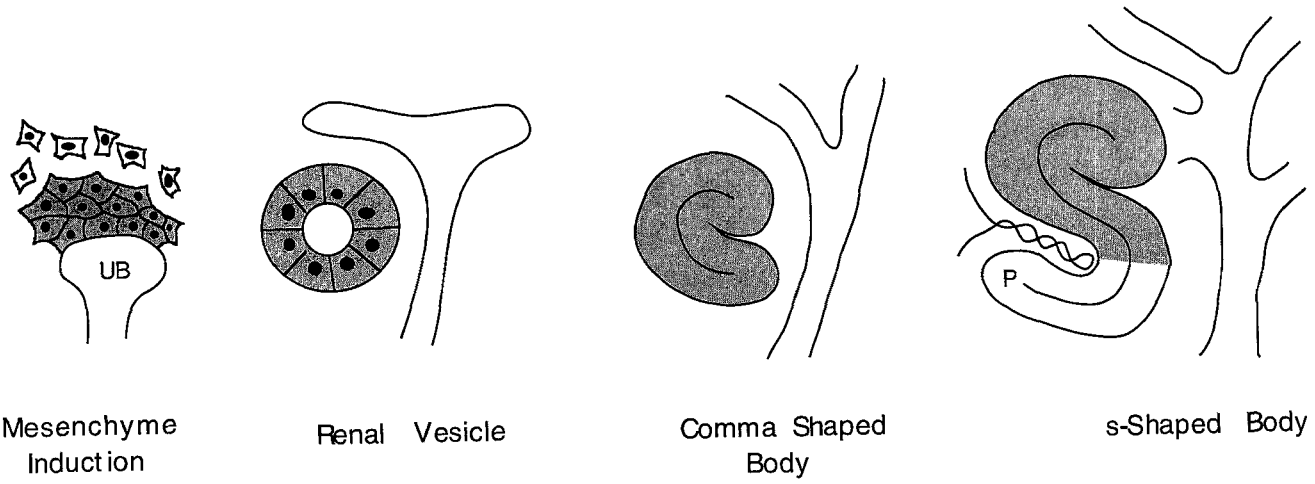

Fig. 1. Expression of *Pax-2* in developing renal epithelium. In the metanephric mesenchyme, *Pax-2* is activated just after induction (shaded cells). These induced cells form aggregates at the tips of the ureteric bud (UB) and convert to a primitive epithelium, the renal vesicle. Meanwhile, the ureteric bud continues to grow and branch further. One side of the renal vesicle invaginates to form the commashaped body. Subsequent extension and convolusions generate the s-shaped body that becomes vascularized in the proximal cleft. Some markers of podocytes, proximal tubule cells, and distal tubule cells are already restricted in the s-shaped body. *Pax-2* expression is down regulated in the podocyte precursor cells (shaded area) where high levels of *WT1* are evident. *Pax-2* is also expressed in the ureteric bud at all stages.

in a panel of RCC cell lines and in primary tumors [19]. These RCC cell lines had mutations or deletions in the *VHL* tumor-suppressor gene [34]. A high proportion of RCC cell lines (73%) expressed *Pax-2,* as did the corresponding primary tumors tested [35]. In the cell lines, *Pax-2* protein synthesis could be inhibited by the addition of antisense oligodeoxynucleotides (ODNs) directed against *Pax-2* mRNA resulting in significant growth inhibition of those cells. Phosphorothioate substituted 19-mers were designed to span either the translation start site or a splice site in the coding region of the mRNA. Similar ODNs inhibited *Pax-2* expression in murine kidney organ cultures [7]. Specific repression of *Pax-2* protein levels could be detected by Western blot analysis over a range of antisense ODN concentrations. The effect of *Pax-2* suppression on cell growth was examined in the RCC lines UOK111 and UOK117, as well as the *Pax-2* negative cell line KN41 and cos-7. Over the first 3 days of culture, antisense *Pax-2* ODNs had no significant growth-inhibitory effects. However, by day 4 the antisense-treated UOK111 and UOK117 cells plateaued and cell numbers actually decreased over the next 2 days. The cell lines not expressing *Pax-2* showed no growth inhibition when cultured with antisense ODNs. The data suggest a role for *Pax-2* in the proliferation of tumor cells and point to the reactivation of this developmentally important gene as a determinant for oncogenesis.

MODEL OF ACTIVATION AND REACTIVATION

The failiure to suppress, or the reactivation of, developmentally specific control genes can potentially predispose a tissue to a number of disease processes. In addition to renal tumors, *Pax-2* expression is also found in regenerating proximal tubule cells after toxic injury [34] and in the proliferating cystic epithelial cells of polycystic kidney [21]. It seems fair to say that *Pax-2* expression is a good marker for proliferating cells derived from the metanephric mesenchyme. Thus, how *Pax-2* is activated in response to induction and how the gene is repressed in terminally differentiated cells may be fundamental for understanding the initiation and progression of a variety of renal diseases.

A current model of *Pax-2* activation and function during renal epithelial cell proliferation is summarized in Figure 2. Activation during normal development requires inductive signals from the ureteric bud. As the epithelial cells proliferate, *Pax-2* expression persists. At a time shortly before, or concurrent with, terminal differentiation, *Pax-2* expression is down-regulated. This repression is most probably mediated by different factors depending on the cell type. Reactivation of *Pax-2* is observed in regenerating proximal tubule cells and may be a response to local cues secreted by injured cells. It is not clear whether the signals mediating *Pax-2* reactivation during regeneration and normal development are similar. One could thus propose that failure to repress *Pax-2* expression following local injury and regeneration might be one determinant for adult RCC. Although tissue culture experiments suggest that *Pax-2* is required at least in some lines for proliferation of RCC cells. It remains to be seen whether tumor growth in vivo is also dependent on *Pax-2*. Nevertheless, the available data point to a striking role for this developmental transcription factor in a number of renal diseases.

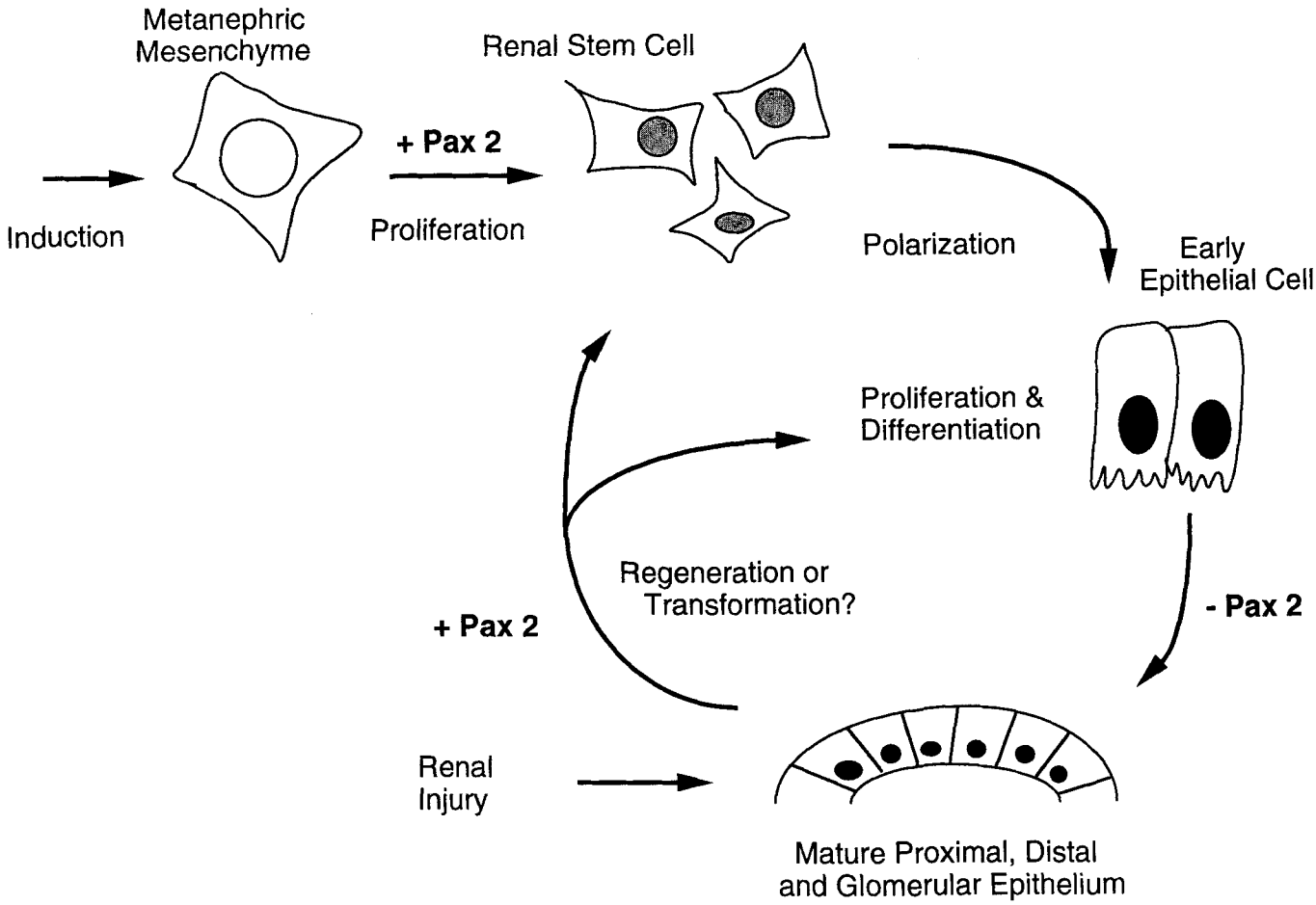

Fig. 2. Summary of *Pax-2* activation and repression during the course of renal epithelial differentiation. Activation in the induced mesenchyme is an early response to induction. Expression persists in the proliferating epithelial cells of the immature nephron. Mature tubule cells repress *Pax-2* but may retain the ability to reactivate expression if subject to renal injury or oncogenic factors.

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COMMENTARY

It is known that the *WT1* protein is capable of repressing *Pax-2,* another gene encoding for a transcription factor playing a role in kidney development, expression during normal kidney development. Here, Dressler proposes that *WT1* gene mutations that alter *WT1* protein production (either decreased production or normal production of abnormal protein) might result in a failure to suppress *Pax-2* and hence uncontrolled cell proliferation. Dressler first describes some characteristics of *Pax-2,* including its expression pattern which is different from that of *WT1. Pax-2* is expressed in the ureteral bud and in the induced kidney mesenchyme harboring the progenitor cells of the glomerular and tubular epithelium. Consequently, *Pax-2* is required in the regulation and differentiation of renal epithelium. Eventually *Pax-2* expression needs to be down-regulated; failure to do so results in severe kidney abnormalities. Based on his observations, both in Wilms' tumor and in RCC, Dressler provides a model for *Pax-2* activation and repression during normal development and reactivation following local injuries. In the last part of the manuscript, the author queries whether failure to repress *Pax-2* expression following local injury could lead to cancer.